Prevalence of Melioidosis in the Er-Ren River Basin, Taiwan: Implications for Transmission

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An increase in melioidosis cases compared to other areas in Taiwan was observed in the Er-Ren River Basin, southwestern Taiwan, from November 2001 to August 2006. The objective of this study was to determine the association between the level of exposure to Burkholderia pseudomallei and the incidence rate of melioidosis and to survey the transmission modes of B. pseudomallei in the Er-Ren River Basin. The serosurveillance of melioidosis gave seropositivity rates of 36.6%, 21.6%, and 10.9%, respectively, for residents in regions A, B, and C within the Er-Ren Basin area. Culture and PCR-based detection of B. pseudomallei from soil demonstrated that the geographical distribution of this bacterium was confined to a particular site in region B. The distribution of seropositive titers was significantly associated with the incidence rate of melioidosis (120, 68, or 36 incidence cases per 100,000 population in region A, B, or C in 2005), whereas it did not correlate with the geographical distribution of B. pseudomallei within the soil. A survey of transmission modes showed that residents with seropositivity were linked to factors such as having confronted flooding and having walked barefoot on soil, which are potential risk factors associated with exposure to B. pseudomallei. Our findings indicated that the Er-Ren River Basin in Taiwan has the potential to become a high-prevalence area for melioidosis. This is the first report that documents a high prevalence of melioidosis in an area north of latitude 20°N.

Melioidosis is a fatal infectious disease caused by Burkholderia pseudomallei, which is endemic in Southeast Asia and northern Australia (7). Human infection with B. pseudomallei usually occurs by inhalation or subcutaneous inoculation and only rarely through ingestion (13). Clinical manifestation includes a variety of symptoms ranging from an unapparent localized chronic infection to a full-blown systemic infection. After the onset of acute septicemia, the mortality rate is about 40% (2). Worldwide, fatal pulmonary melioidosis has been increasing among travelers returning from areas of endemicity (8).

B. pseudomallei is a saprophyte, and it is widely distributed in tropical soil and water but with an uneven distribution (13). The presence of B. pseudomallei in soil is associated to some degree with areas having a high incidence of melioidosis (23, 24, 27). Most patients with melioidosis in Thailand are farmers who suffer from heavy exposure to B. pseudomallei during agricultural activities (22). Human exposure to B. pseudomallei may occur at preschool ages, as serosurveillance in northeast Thailand shows (25). In areas of nonendemicity, the seropositivity rate is relatively low because individuals have little chance to come into contact with the pathogen (5). However, there has been no systematic international study of seroprevalence rates using a consistent, standardized, and quality-controlled serological test. It is thus difficult to make adequate comparisons between countries and regions.

Melioidosis in Taiwan was first reported in 1984 when a traveler was diagnosed as having a pulmonary infection after a drowning incident near Manila, Philippines (18). Since 1994, cases of melioidosis in Taiwan have been steadily increasing and have appeared to be indigenous as these patients had never traveled overseas (3, 16, 19, 20). The clinical manifestations of melioidosis are quite protean, and therefore clinical diagnosis is often difficult. As a result, the true incidence of melioidosis may be higher than is currently recognized (12). Whether this increase in the diagnosis of melioidosis in Taiwan is due to the widespread existence of B. pseudomallei or a previous underdiagnosis of cases remains unanswered.

We have previously demonstrated that B. pseudomallei can be isolated from cropped soil in southern Taiwan (17) and that city-dwelling individuals have a seropositivity rate of only 5% for melioidosis (5). It appears that specific antibodies against B. pseudomallei can still exist in humans even 6 months after exposure (6). The cases of melioidosis that occurred in the Er-Ren River Basin in 2005 represent the highest incidence rate of melioidosis among all regions in Taiwan. Thus, we conducted an epidemiological investigation to determine the seroprevalence rate and geographical distribution of B. pseudomallei in the Er-Ren River Basin.
Soil sampling. Soil samples were collected from various cropped fields that were located on both sides of the main Er-Ren River and its branches. The sampling sites were separated by between 0.5 km and 1 km and stretched from 2 sites/km². Each site was sampled from three separate holes at the same time. Approximately 100 g of soil sample was obtained using a sterile tube. PCR detection. The genomic DNA of bacteria present in the soil was isolated using another kit (IsoQuick; ORCA Research, Inc.). Two primer sets (16SrRNA gene, forward, 5′-CGGAGCAGGCAGCCTTCG-3′; reverse, 5′-CGGAGCCGGCCTTCG-3′; 16S rRNA gene, forward, 5′-CTGCGTGCTGCGCTGGCCG-3′; reverse, 5′-CTGCGTGCTGCGCTGGCCG-3′) were used to amplify specific-amplification products (243 and 405 bp for the 16S rRNA gene and 267 bp for the flagella gene) (14, 17). The PCR mixture consisted of 1 pg genomic DNA, 0.5 μmol of each primer, 250 μmol/liter deoxynucleotide triphosphate (dNTP), 1× PCR buffer, and 1 U Taq polymerase with a final volume of 50 μl. The PCR profile consisted of 40 cycles of 1 min at 94°C, 30 s at 60°C, and 1 min at 72°C, with a final extension step of 10 min at 72°C. The products were visualized using agarose electrophoresis. When amplicons of both the 16S rRNA gene and the flagella gene were observed, the sample was considered to be positive for B. pseudomallei (17).

Results. From November 2001 to August 2006, 54.1% (72/133) of melioidosis cases in Taiwan occurred in the Er-Ren River Basin in southwestern Taiwan (Fig. 1). Detailed information on the incidence rate of melioidosis in Taiwan, the Er-Ren River Basin, and the various regions within the Er-Ren River Basin are listed in Table 1. The Er-Ren River Basin had an incidence of 100,000 in 2005, the highest incidence rate compared in Taiwan. We set out to determine if residents in the Er-Ren River Basin were exposed to B. pseudomallei. Therefore, 624 serum samples were collected from residents to detect the presence of specific antiflagellin antibodies. The Er-Ren River Basin can be divided into three regions based on the seropositivity rate, namely high (A [36.6%]), medium (B [21.6%]), and low (C [10.9%]) (Table 2). The seropositivity rate in the Er-Ren River Basin was associated with the incidence of melioidosis as follows: 120/100,000 in region A, 68/100,000 in region B, and 36/100,000 in region C (Table 1). If each region was subdivided into several sites (A1 and A2; B1, B2, and B3; and C1, C2, and C3) based on the presence of geographical barriers such as river segmenting or discontinuous locations (Fig. 1), then the association between seropositivity and incidence rate was not sustained for each subdivi- sion (Table 1). Sites B3 and C3 exhibited seropositivity rates among the residents of 23.8% and 9.2%, respectively (Table 2), although these sites did not report melioidosis cases in 2005.

To determine if the geographical distribution of B. pseudomallei was linked to seropositivity in residents in the Er-Ren River Basin, bacterial isolation combined with PCR-based detection were used to detect the presence of B. pseudomallei in soil samples. The results indicated that the geographical distribution of culturable B. pseudomallei was confined to site B3, where there was a 26.4% positive bacterial isolation rate for B. pseudomallei (Table 2). Nevertheless, the presence of B. pseudomallei was detected over an extensive region using the PCR-based technique. There were positivity rates of 3.1% to 6.7%, 2.6% to 33.0%, and 0% to 5.6% in regions A, B, and C, respectively (Table 2). However, the actual incidence of melioidosis (Table 1) and the seropositivity rate for B. pseudomallei (Table 2) did not correlate with the presence of B. pseudomallei in soil from the Er-Ren River Basin in these areas.

To address the possible transmission modes of B. pseudomallei in the Er-Ren River Basin, a survey was conducted to evaluate the daily routine of the residents. Variables including sex, age, travel history, and occupation were first excluded from these variables did not show statistical significance. Being barefoot was a significant factor (29.9%; P < 0.05), as reported.
from region A (Table 3), where there was a 36.6% seropositivity rate for residents (Table 2). In addition, experiencing flooding within the last 6 months was also a significant factor reported from region A (32.8%; \( P < 0.05 \)) and B (34.2%; \( P < 0.05 \)) (Table 3). Only 13.4% of residents complained of this flooding in region C, where there was only a 10.9% seropositivity rate for residents. It seems that walking barefoot and flooding are important factors that might result in a high risk of exposure to \( B. \) pseudomallei in region A. The same questionnaire was given to 30 patients who had contracted melioidosis in the last 6 months, with a response rate of 43% (13/30). The results showed that 38.5% were farmers and 46.2% were living near fields, but only 7.7% had walked barefoot on the soil. It is interesting that 38.5% of respondents had experienced a flood within the past 6 months. It appears that water contact is the most important factor contributing to infection with \( B. \) pseudomallei among residents in the Er-Ren River Basin.

**DISCUSSION**

Melioidosis in Taiwan has been recognized as an emerging disease (16). However, its prevalence has not yet been fully evaluated despite a substantial increase in sporadic cases over recent years (3, 16, 19, 20). Data from 2001 to 2006 show that cases of melioidosis are not evenly distributed; 54.1% of cases were localized to the Er-Ren River Basin in southwestern Taiwan. We have confirmed that residents in the various regions within Er-Ren River Basin exhibited 10.9% to 36.6% seropositivity for melioidosis, which is significantly higher than the 2.5% to 5% that has been reported among Taiwanese in

**TABLE 1. Summary of the annual incidence of melioidosis in Taiwan in 2005**

<table>
<thead>
<tr>
<th>Location</th>
<th>Incidence rate/100,000 population (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taiwan</td>
<td>0.3 (0.25–0.40)</td>
</tr>
<tr>
<td>Er-Ren River Basin</td>
<td>70 (49.49–89.69)</td>
</tr>
<tr>
<td>Region and subdivision</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>120 (71.10–169.29)</td>
</tr>
<tr>
<td>A1</td>
<td>60 (7.44–60.12)</td>
</tr>
<tr>
<td>A2</td>
<td>166 (89.59–166.39)</td>
</tr>
<tr>
<td>B</td>
<td>68 (31.33–105.91)</td>
</tr>
<tr>
<td>B1</td>
<td>73 (1.49–73.33)</td>
</tr>
<tr>
<td>B2</td>
<td>124 (43.14–124.30)</td>
</tr>
<tr>
<td>B3</td>
<td>ND</td>
</tr>
<tr>
<td>C</td>
<td>36 (13.57–57.80)</td>
</tr>
<tr>
<td>C1</td>
<td>55 (7.25–55.25)</td>
</tr>
<tr>
<td>C2</td>
<td>52 (51.99–213.49)</td>
</tr>
<tr>
<td>C3</td>
<td>ND</td>
</tr>
</tbody>
</table>
| a Data are shown as the incidence rate per 100,000 population, with 95% confidence intervals in parentheses. ND, no confirmed case found.

**TABLE 2. Summary of the seroprevalence and geographical distribution of \( B. \) pseudomallei in the Er-Ren River Basin**

<table>
<thead>
<tr>
<th>Region and subdivision</th>
<th>Seroprevalence*</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sampling size (n)</td>
<td>% Seropositive</td>
</tr>
<tr>
<td>A</td>
<td>183</td>
<td>36.6</td>
</tr>
<tr>
<td>A1</td>
<td>37.1</td>
<td>36.6</td>
</tr>
<tr>
<td>A2</td>
<td>35.6</td>
<td>36.6</td>
</tr>
<tr>
<td>B</td>
<td>176</td>
<td>21.6</td>
</tr>
<tr>
<td>B1</td>
<td>23.1</td>
<td>21.6</td>
</tr>
<tr>
<td>B2</td>
<td>19.5</td>
<td>21.6</td>
</tr>
<tr>
<td>B3</td>
<td>23.8</td>
<td>21.6</td>
</tr>
<tr>
<td>C</td>
<td>265</td>
<td>10.9</td>
</tr>
<tr>
<td>C1</td>
<td>11.1</td>
<td>10.9</td>
</tr>
<tr>
<td>C2</td>
<td>9.2</td>
<td>10.9</td>
</tr>
<tr>
<td>C3</td>
<td>ND</td>
<td>10.9</td>
</tr>
</tbody>
</table>
| a Significance (\( P < 0.05 \)), A > B > C.
general (5). Moreover, environmental samples that yielded
positive PCR or culture results demonstrated the presence of
*B. pseudomallei* in the cropped soil in the Er-Ren River Basin.
Taken together, the Er-Ren River Basin in Taiwan should be
described as a high-risk region for the occurrence of a number of
melioidosis cases.

Early classification of *B. pseudomallei* environmental isolates
divided them into pathogenic (arabinose nonassimilating) and
nonpathogenic (arabinose assimilating) strains. However, non-
pathogenic strains are now classified as *Burkholderia thailand-
densis* (1) and are easily distinguished from the pathogenic
strains based on specific 16S RNA gene and flagellum gene
amplicons (14, 21). Using the presence of these specific amp-
licons as detected by a PCR-based technique, it was demon-
strated that *B. pseudomallei* existed in a wide range of soil
samples. However, it was sometimes not possible to enrich
these PCR-positive soil specimens to allow isolation of *B.
pseudomallei* in this study. This may be because a cross-reac-
tion of PCR occurred between *B. pseudomallei* and other un-
identified organisms. In addition, the presence of nonviable or
unculturable *B. pseudomallei* could also result in negative re-
sults for the bacterial cultures. Since we have previously dem-
onstrated *B. pseudomallei* is capable of survival and growth in
soil media mimicking the Taiwan environment for 6 months
(4), a very low level of bacteria or the presence of unculturable
bacteria might be the origin of the infection risk. This risk
would occur when conditions that restrict the growth of *B.
pseudomallei* are removed and the organism becomes more
prevalent.

**B. pseudomallei** inhabits soil or water in tropical areas and in
particular is found between latitudes 20°N and 20°S (7). Its
geographical distribution is uneven. For example, the isolation
rate was 37% in Pattalung but only 3% in Trang, neighboring
provinces in southern Thailand (15). In this study, the isolation
rate from site B3 was high at 26.4%. Beyond this site, the distribu-
tion of *B. pseudomallei* was low, although the presence of
the bacteria was occasionally detected by PCR. This indi-
cates that site B3 is a potential risk area that may be involved
in spreading this bacterium across the Er-Ren River Basin.
This is the first reported area with such a high isolation rate for
*B. pseudomallei* north of latitude 20°N.

In this study, the order of the seropositive titers of the
various regions correlated with the degree of the incidence rate
of melioidosis, but there was no direct correlation with the
presence of viable *B. pseudomallei* at specific sites within the
Er-Ren River Basin. Since the geographical distribution of *B.
pseudomallei* is usually uneven, the transmission of the infec-
tious organism causing melioidosis from one site to another
has been proposed to occur through vectors such as floodwater
or wind (10). In one particular instance, during an outbreak of
melioidosis in Australia, it was found that the disease was
spread by a water conduit (11). Based on our data, 32.8% or
42.3% of residents shared common experiences of flooding in
regions A or B. The seropositivity and incidence rates for
melioidosis in both regions were significantly higher than in
region C. It is possible that residents were infected by the
bacterium through floodwater. Alternatively, there could have
been direct contact with propagating unculturable bacteria that
had rapidly proliferated under suitable growth conditions.

The annual incidence of melioidosis in the Er-Ren River
Basin in 2005 was 70/100,000, which is higher than those of
areas where melioidosis is endemic: for example, 16.5/100,000
in the top end of the Northern Territory, Australia, and 4.4/
100,000 in Ubon Ratchathani Province in northeast Thailand
(7). Indeed, the presence of *B. pseudomallei* in soil and a high
seropositivity rate in residents were clearly evident for the
Er-Ren River Basin. Physicians who practice in this area
should be aware of melioidosis when patients present with an
unknown fever or community-acquired pneumonia. Special at-
tention should be given to seropositive individuals because *B.
pseudomallei* may persist in humans for a long time and can
easily reactivate, which can lead to patient relapse, especially
among immunocompromised individuals, in whom the disease
can be fatal (7, 9).

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**REFERENCES**


