The gram-negative soil-dwelling saprophytic bacterium *Burkholderia pseudomallei* causes melioidosis, a fatal disease highly endemic to Southeast Asia and northern Australia (1). Humans can be infected with *B. pseudomallei* via inoculation, inhalation, and ingestion. Rice farmers are at high risk for infection because of their frequent exposure to soil and water, but newborns, children, and older persons also are at risk (2,3). We report 3 melioidosis deaths among children in northern Vietnam.

The Study

In November 2019, the Preventive Health Center of Soc Son district in Vietnam reported the deaths of 3 children from 1 family. The first child, a 7-year-old girl, had a high fever and abdominal pain on April 6, 2019. Two days later, she was admitted to a local hospital; after 1 day, she was transferred to St. Paul Hospital in Hanoi, where septic shock was diagnosed. She died on April 9, shortly after admission, before any diagnostic tests were performed.

On October 27, 2019, the second child, a 5-year-old boy, had a high fever and abdominal pain around the umbilicus. He was admitted to Vietnam National Children’s Hospital in Hanoi on October 28 with diagnosed septic shock. Abdominal and chest radiographs and abdominal ultrasound results were unremarkable. His blood culture grew *B. pseudomallei*, and he died on October 31.

The third child, a 13-month-old boy, had a high fever and poor appetite on November 10, 2019. According to his grandparents, he had black stool, like his sister and brother. He was admitted to Vietnam National Children’s Hospital; chest radiographs results were unremarkable, but *B. pseudomallei* was cultured from his blood sample. He died on November 16.

We retrieved laboratory findings from all hospitals to which these children were admitted. Results showed leukopenia, neutropenia, thrombocytopenia, and high procalcitonin and C-reactive protein in all children’s blood. Liver dysfunction was diagnosed in all 3 children, but kidney dysfunction was recognized only in the 2 older children. We detected no identifiable risk factors (Table 1).

To trace the source of infection, on November 17, 2019, we visited the family home in the midland region of northern Vietnam (Figure 1). During our active surveillance for melioidosis cases admitted to provincial and tertiary hospitals surrounding Hanoi (4), no previous cases had been reported from this area.

We interviewed the parents and grandparents using epidemiologic questions about all the children’s daily activities inside and outside the house. The family used water supplied from 3 boreholes: 1 for bathing (borehole A), 1 for livestock (borehole B), and 1 for human consumption (borehole C). During our first environmental investigation, we collected samples of front garden soil (n = 7), borehole water (n = 9), and boiled drinking water (n = 1). We performed qualitative culture for *B. pseudomallei*, and all 3 water samples collected from borehole A tested positive (Appendix, https://wwwnc.cdc.gov/EID/article/28/8/22-0113-App1.pdf).
We revisited the home on November 23, 2019, and asked the family about the history of borehole A. In brief, the borehole was drilled in 2010. In 2015, the family reconstructed the back garden and added a new soil layer, resulting in the bore cap being ≈80 cm below the soil surface (Figure 2, panel A). At the end of 2018, the foot valve in the suction pipe of the dynamic electric pump was damaged, and the bore cap was not sealed after the damage was repaired (Figure 2, panel B). We suspected rainwater and surface soil particles contaminated with B. pseudomallei drained into the groundwater via the opened borehole. To test this hypothesis, we conducted a second round of environmental sampling, focusing on borehole A and the nearby surface soil. We collected 26 borehole water and 46 garden soil samples. Within a 1-km radius of the home, we also collected 39 water samples from other boreholes, 30 surface water samples from 10 ponds, and 40 soil samples from 8 rice fields (Figure 1; Appendix).

We found 26 (100%) water samples collected from borehole A and 27 (58.7%) garden soil samples from 8 (80%) sampling points near the borehole were B. pseudomallei-positive by qualitative culture. These findings supported our hypothesis that B. pseudomallei from surface soil might have contaminated the groundwater through the unsealed bore cap during the rainy season, which starts in April and coincided with the first child’s illness and death. Another 5 (12.5%) soil samples from 2 (25%) rice fields also tested B. pseudomallei-positive. Quantitative culture showed that the median B. pseudomallei count was 406 CFU/g (range 12–746 CFU/g) in soil (Appendix). Of 26 water samples collected from borehole A, 2 (7.7%) grew B. pseudomallei on the initial agar plates and had a median B. pseudomallei count of 2 CFU/mL (Table 2).

We selected 20 B. pseudomallei isolates for multilocus sequence typing (MLST) (5): 7 from borehole A, 6 from back garden soil, 5 from rice field soil,
B. pseudomallei – Contaminated Borehole Water

and 2 from blood samples from cases 2 and 3. MLST showed an identical sequence type (ST), 541, among all samples (Table 2).

Conclusions

B. pseudomallei is ubiquitously distributed in soil and surface water throughout the tropics, including in Asia, the Pacific Islands, sub-Saharan Africa, and Latin America, where boreholes are the most common water supply in the rural areas (1,6,7). In addition to other waterborne infections (7), untreated water supplies have been implicated in previous human B. pseudomallei infections (8–10). B. pseudomallei also was isolated from the compacted earth floor under the bathing tub of a woman who died from septicemic melioidosis in Brazil (11).

Studies in Australia and Thailand detected diverse STs among B. pseudomallei isolates from an unchlorinated bore water site and a single soil sample (12,13), but our analysis revealed a single ST in the borehole, nearby garden, and surrounding rice fields. Because all 3 infections occurred in children, we believe B. pseudomallei transmission likely occurred through ingestion of contaminated water during bathing, especially considering that the 13-month-old boy was not in contact with garden or rice field soil. Ingestion also could explain the gastrointestinal symptoms the children exhibited.

Figure 1. Environmental sampling sites in an investigation of 3 child deaths from melioidosis caused by Burkholderia pseudomallei–contaminated borehole water, Vietnam, 2019. The satellite map was created using QGIS software version 3.22.1 (https://www.qgis.org). Red outline indicates the family property where the children lived; red circle is borehole A from which B. pseudomallei was isolated. Yellow outlines are rice fields from which soil samples were collected; red stars indicate rice fields that tested positive for B. pseudomallei. Yellow circles indicate neighbors’ boreholes and yellow squares indicate neighbors’ ponds from which water samples were collected. Inset map shows Vietnam; red square indicates sampling area.

Figure 2. Borehole involved in 3 child melioidosis deaths caused by Burkholderia pseudomallei–contaminated borehole water, Vietnam, 2019. A) View of area around borehole. The bore cap is ~80 cm below the soil surface inside the masonry area. Red arrow indicates cracks in the masonry construction that might enable rainwater and soil particles to drain into the borehole area. B) View from above the borehole. Red arrow indicates the unsealed, opened gap around the borehole, which likely enabled rainwater and soil particles to drain into the groundwater during the rainy season.
ST541 died, which could mean... and other pathogens from... 14–16... during the investigation. B. pseudomallei and sincerely thank them for providing information. We express our deepest condolences to the family members.

**Acknowledgments**

We express our deepest condolences to the family members and sincerely thank them for providing information during the investigation.

_B. pseudomallei_ ST541 has been reported from human melioidosis cases in northern Vietnam (3) and has only been described from southeast Asia thus far. During previous surveillance (4), we found other _ST541_ isolates in clinical and environmental samples from north and north-central Vietnam. An _ST541_ isolate available in a public MLST database (https://pubmlst.org/organisms/burkholderia-pseudomallei; accessed 2021 Dec 8) was from a human case in Hainan, China, which is close to the area of Vietnam where these 3 melioidosis deaths occurred. From our clinical data retrieval (3,4), 5 of 8 patients infected with _B. pseudomallei_ ST541 died, which could mean _ST541_ is more virulent than other STs, but further study is needed.

From the epidemiologic investigation and field study at the family home, we became aware of the construction and maintenance of the borehole, which had an unsealed cap and an open borehole below the soil surface. The unsealed borehole probably enabled _B. pseudomallei_ from surface soil to contaminate groundwater during rainfall. Other studies have reported higher rates of gastrointestinal pathogens in water from boreholes with unsealed annuli (14,15). Therefore, persons using boreholes in countries where melioidosis is endemic should ensure proper construction and maintenance to avoid contamination with _B. pseudomallei_ and other pathogens from surface soil.

**References**


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Child Melioidosis Deaths Caused by \textit{Burkholderia pseudomallei}–Contaminated Borehole Water, Vietnam, 2019

Appendix

Materials and Methods

Soil Sampling

A primary environmental investigation was conducted at the family property on November 17, 2019. Seven soil samples at a depth of 10 cm were collected at different sampling points of the front garden. Nine borehole water (3 samples from each borehole) and one boiled drinking water samples were also collected.

A secondary environmental sampling was conducted on November 23, 2019. At borehole A, the electric pump was turned on, and 26 bore water samples were collected every five minutes (from 0 to 120 minutes) and at 240 minutes. Forty-six soil samples from 10 sampling points near borehole A in the back garden were also collected, and the distance between each sampling point was \(\approx 10\) m. At each point, the soil samples were collected at depths of 10, 20, 30, 40, and 50 cm, except for 2 points where only soil samples at depths of 10, 20, and 30 cm were collected. Additionally, 39 bore water samples were collected from the other two boreholes on the family property and 11 boreholes in the neighborhood (3 samples from each borehole). Thirty surface water samples from 10 surrounding ponds (3 samples from each pond) were collected. Forty soil samples from eight rice fields were collected at a depth of 30 cm (5 samples from each rice field) (Figure 1).

Qualitative Culture of \textit{B. pseudomallei}

Detection of \textit{B. pseudomallei} from water and soil samples was performed using a two-step enrichment approach (1). In brief, a 10-gram soil sample was added to 50 mL tubes containing 20 mL of TBSS-C50 broth. After vigorous vortexing, the tubes were statically
incubated at 40°C for 2 days. Subsequently, 1 mL of the culture supernatants were transferred to new tubes containing 9 mL of MB broth. After static incubation at 40°C for 4 days, the enriched culture supernatants were streaked out on Ashdown agar plates. The plates were then incubated at 40°C for 4 days and examined every day. Based on morphological characteristics, suspected colonies of \textit{B. pseudomallei} were picked up, and the bacterial identification was confirmed using the \textit{B. pseudomallei}-specific real-time PCR assay targeting the TTSS1 gene (2). The bacterial isolates were stored at −70°C in Luria-Bertani broth containing 20% glycerol for further genotype experiment.

For water samples, 100 mL of water sample was centrifuged at 5,000 rpm for 30 min, and the supernatants were decanted to obtain the water sediments. Then, 20 mL TBSS-C50 broth was added to the tubes, and the culture approach for \textit{B. pseudomallei} was performed, as described above.

\textbf{Quantitative Culture of \textit{B. pseudomallei}}

The bacterial count was only performed on water or soil samples positive for \textit{B. pseudomallei} by the quantitative culture. In brief, a 10-gram soil sample was added to a 250-mL Erlenmeyer flasks containing 20 mL of distilled water. The soil was dispersed by shaking at 160 rpm for 2 h at room temperature. The flasks were left for 30 min to allow the soil particles settle. Then 100 µL of the upper layer suspension and its serial 10-fold dilutions were plated out on the Ashdown agar plates. After incubation at 40°C for 4 days, the suspected \textit{B. pseudomallei} colonies were counted, and the CFU/g of soil was calculated, as previously described (3).

For the water samples, 500 µL of borehole A water was plated out on the Ashdown agar plates. After incubation at 40°C for 4 days, the suspected \textit{B. pseudomallei} colonies were counted, and the CFU/mL of water was calculated.

\textbf{Physiochemical Parameters of Soil and Water Samples}

Compared with \textit{B. pseudomallei}–negative borehole water samples, physicochemical parameters showed water samples from borehole A had low pH and high nitrate, iron, total suspended solids, and total organic carbon (Appendix Table 1). \textit{B. pseudomallei}–positive soil collected in the garden near borehole A had much lower electrical conductivity but much higher total potassium oxide and aluminum levels than \textit{B. pseudomallei}–negative soil samples (Appendix Table 2).
References


**Appendix Table 1.** Physicochemical parameters of water samples from 1 *Burkholderia pseudomallei*-contaminated borehole and 13 other boreholes investigated in the deaths of 3 children from melioidosis, Vietnam, 2019*

<table>
<thead>
<tr>
<th>Physicochemical parameters, mg/mL</th>
<th>Contaminated borehole</th>
<th>Other boreholes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.91</td>
<td>6.13 (3.98–7.19)</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>2.47</td>
<td>1.35 (0.85–2.67)</td>
</tr>
<tr>
<td>Ammonium</td>
<td>ND</td>
<td>0.14 (ND–0.4)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>4.80</td>
<td>3.08 (0.22–4.54)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>ND</td>
<td>0.21 (ND–0.23)</td>
</tr>
<tr>
<td>Iron</td>
<td>0.34</td>
<td>0.19 (0.11–0.30)</td>
</tr>
<tr>
<td>Total organic carbon</td>
<td>4.70</td>
<td>3.31 (2.50–4.20)</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>6.00</td>
<td>5.50 (4.00–8.00)</td>
</tr>
<tr>
<td>Biologic oxygen demand</td>
<td>1.00</td>
<td>0.75 (0.00–2.00)</td>
</tr>
</tbody>
</table>

*Data represent mean (range); ND, not detected.

**Appendix Table 2.** Physicochemical parameters of soils collected from the garden and rice fields investigated for *Burkholderia pseudomallei* contamination in the deaths of 3 children from melioidosis, Vietnam, 2019*

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Garden soil</th>
<th>Rice field soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH&lt;sub&gt;KCl&lt;/sub&gt;, KCl, mmol/L</td>
<td>3.73 ± 0.16</td>
<td>4.08 ± 0.24</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>14.30 ± 2.07</td>
<td>16.50 ± 3.07</td>
</tr>
<tr>
<td>Electrical conductivity, µS/cm</td>
<td>75.48 ± 25.93</td>
<td>105.50 ± 53.17</td>
</tr>
<tr>
<td>Organic carbon, %</td>
<td>0.99 ± 0.19</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Total nitrogen, %</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Total P&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;, %</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Iron, g/Kg</td>
<td>2.18 ± 0.52</td>
<td>2.15 ± 1.31</td>
</tr>
<tr>
<td>Total K&lt;sub&gt;2&lt;/sub&gt;O, %</td>
<td>1.48 ± 0.49</td>
<td>0.96 ± 0.17</td>
</tr>
<tr>
<td>Aluminum, g/Kg</td>
<td>4.75 ± 0.59</td>
<td>2.27 ± 1.35</td>
</tr>
</tbody>
</table>

*Data represent mean ± SD; p values were obtained from t-test. K<sub>2</sub>O, potassium oxide; KCl, potassium chloride; P<sub>2</sub>O<sub>5</sub>, phosphorus pentoxide; S, siemens.